Supporting Information for

Reduction/pH Dual-Responsive Nano-Prodrug Micelles for Controlled Drug Delivery

Determination of the calibration curves of DOX in phosphate buffer saline (PBS, pH 7.4, 5.8, 5.0)

Calibration curves of DOX in PBS (0.01M, pH = 7.4, 5.8) and acetate buffer solution (0.01 M, pH = 5.0) were determined by measuring the absorption of DOX with known concentrations via Shimadzu UV2550 UV-vis spectrophotometer at a wavelength of 479 nm, which is the typical absorption for DOX. The absorption as a function of DOX concentration was recorded to generate the calibration curve, which is shown in Fig. S1.

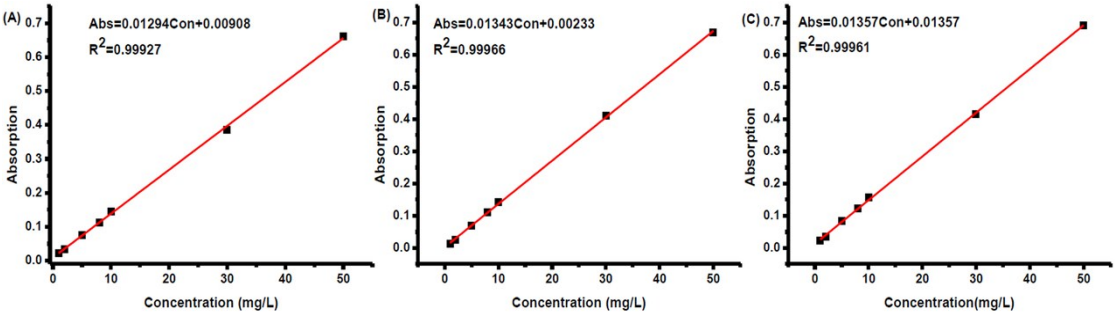

Fig. S1 Calibration curves of DOX in PBS (0.01 M, pH = 7.4) (A), PBS (0.01 M, pH = 5.8) (B) and acetate buffer solution (0.01 M, pH = 5.0).

Determination of the molecular weight and molecular weight distribution of POEGSSFM by GPC

The molecular weight and molecular weight distribution of the obtained POEGSSFM were measured by gel permeation chromatography (GPC), on a Waters degasser, a Waters 1515 Isocratic HPLC pump, and columns: PLgel 5 μm MIXED-C, 300 × 7.5 mm. THF was used as the mobile phase with a flow rate of 1.0 mL/min at 60 °C and standard narrow PDI polystyrene (PS) was used for calibration, as shown in Fig. S2.

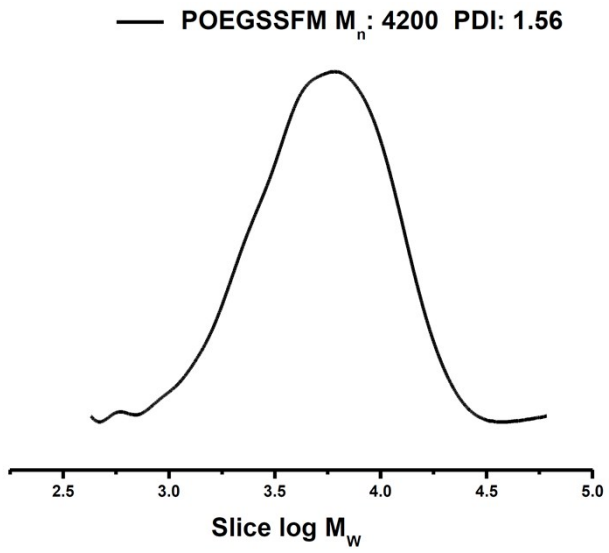

Fig. S2 GPC curve of POEGSSFM.

Preparation and characterization of POEGSSFM micelles

POEGSSFM (25 mg) was dissolved in 5 mL of THF, then 5 mL of PBS (0.01 M, pH 7.4) was added dropwise to the solution under vigorous stirring. The resulting solution was stirred for 2 h and dialyzed (MWCO 3500, Fisher Scientific) against PBS (0.01 M, pH 7.4) over 24 h to completely remove THF and form micelles. POEGSSFM micelles in DMF were obtained by dialysis against DMF. The size of POEGSSFM micelles in aqueous solution and DMF were monitored by DLS measurement. As shown in Fig. S3, the diameter of the micelles was 61 nm with narrow distribution, while no signal of any aggregation could be detected in DMF.

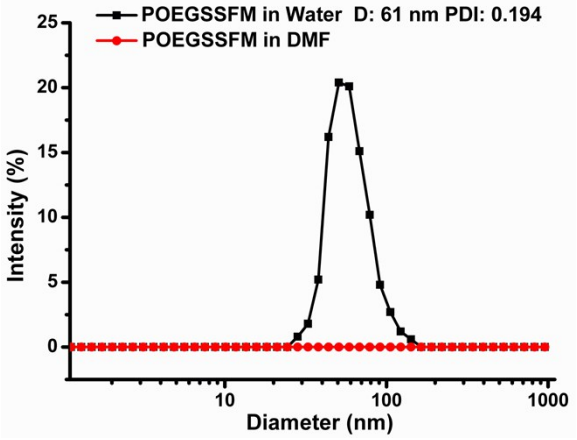

Fig. S3 Particle size and distribution of POEGSSFM by DLS in water and DMF

pH-response of CCL-POEGSSFM-DOX micelles

The size change of CCL-POEGSSFM-DOX micelles in response to acidic conditions was measured by DLS. 2 mL of CCL-POEGSSFM-DOX micellar solution were prepared in pH 5.8 and 7.4 PBS solutions, respectively. The size of the micelles was measured at 0 h, 9 h, and 24 h. As shown in Fig. S4, a slight increase in diameter (from 65 to around 85 nm) was observed for the micelles in pH 5.8 PBS solution after treating for 24 h, while the diameter of micelles in pH 7.4 PBS solution did not show significant change.

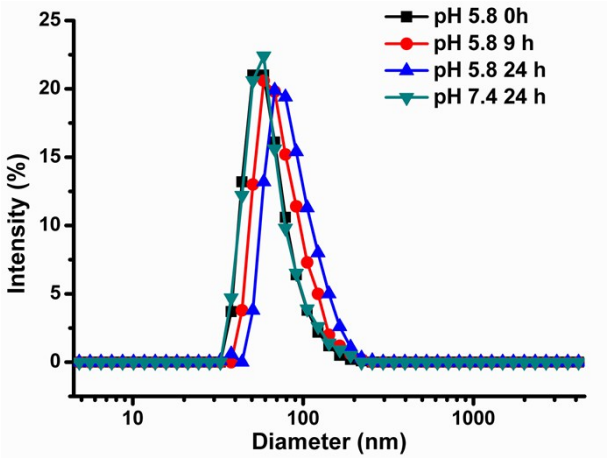

Fig. S4 The size change of CCL-POEGSSFM-DOX micelles in response to pH determined by DLS measurement

***In vitro* cytotoxicity of CCL-POEGSSFM**

The cytotoxicity of CCL-POEGSSFM micelles against MC3T3-E1 cells (Cell bank of the Chinese Academy of Science, China) were evaluated by CCK-8 (Dojindo, Japan) assay. The cells were pre-incubated in a 96-well plate (5×10^3 cells/well) with culture medium 10% FBS/α-MEM (Invitrogen Co., Carlsbad, CA) in a humidified 5% CO_2-containing atmosphere at 37 °C for 24 h. Then incubated with CCL-POEGSSFM with determined concentrations (0.01 mg/L-1000 mg/L) for 48 h. Media was aspirated and replenished with 100 μL of fresh culture medium. 10 μL CCK-8 reagents were added into each well, and the cells were incubated at dark for another 1 h. The absorbance at a wavelength of 450 nm of each well was measured using a microplate reader (Sunrise™ Basic;

TECAN, Zurich, Switzerland). Non-treated cells were used as a negative control, wells without cell but culture medium was used as blank. The results presented in Fig. S5 clearly indicate that the blank micelles without drug moieties are biocompatible to normal tissues.

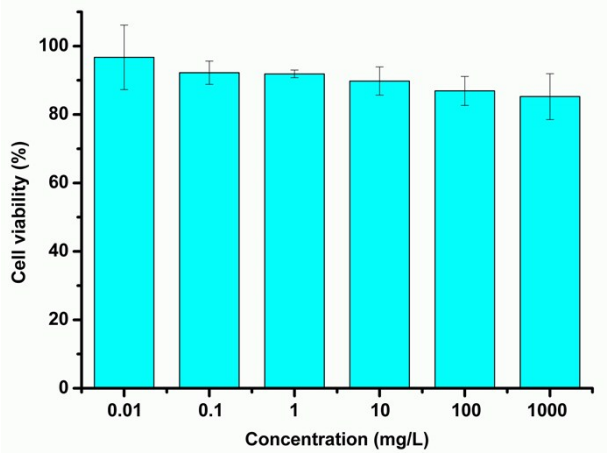

Fig. S5 Cytotoxicity studies of blank CCL-POEGSSFM micelles against MC3T3-E1 cells after incubation for 48 h.